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Long-term light grazing does not change soil organic carbon stability and stock in biocrust layer in the hilly regions of drylands

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Abstract: Livestock grazing is the most extensive land use in global drylands and one of the most extensive stressors of biological soil crusts (biocrusts). Despite widespread concern about the importance of biocrusts for global carbon (C) cycling, little is known about whether and how long-term grazing alters soil organic carbon (SOC) stability and stock in the biocrust layer. To assess the responses of SOC stability and stock in the biocrust layer to grazing, from June to September 2020, we carried out a large scale field survey in the restored grasslands under long-term grazing with different grazing intensities (represented by the number of goat dung per square meter) and in the grasslands strictly excluded from grazing in four regions (Dingbian County, Shenmu City, Guyuan City and Ansai District) along precipitation gradient in the hilly Loess Plateau, China. In total, 51 representative grassland sites were identified as the study sampling sites in this study, including 11 sites in Guyuan City, 16 sites in Dingbian County, 15 sites in Shenmu City and 9 sites in Ansai District. Combined with extensive laboratory analysis and statistical analysis, at each sampling site, we obtained data on biocrust attributes (cover, community structure, biomass and thickness), soil physical-chemical properties (soil porosity and soil carbon-to-nitrogen ratio (C/N ratio)), and environmental factors (mean annual precipitation, mean annual temperature, altitude, plant cover, litter cover, soil particle-size distribution (the ratio of soil clay and silt content to sand content)), SOC stability index (SI) and SOC stock (SOCS) in the biocrust layer, to conduct this study. Our results revealed that grazing did not change total biocrust cover but markedly altered biocrust community structure by reducing plant cover, with a considerable increase in the relative cover of cyanobacteria (23.1%) while a decrease in the relative cover of mosses (42.2%). Soil porosity and soil C/N ratio in the biocrust layer under grazing decreased significantly by 4.1%–7.2% and 7.2%–13.3%, respectively, compared with those under grazing exclusion. The shifted biocrust community structure ultimately resulted in an average reduction of 15.5% in SOCS in the biocrust layer under grazing. However, compared with higher grazing (intensity of more than 10.00 goat dung/m^2), light grazing (intensity of 0.00–10.00 goat dung/m^2 or approximately 1.20–2.60 goat/(hm^2•a)) had no adverse effect on SOCS. SOC stability in the biocrust layer remained unchanged under long-term grazing due to the offset between the positive effect of the decreased soil porosity and the negative effect of the decreased soil C/N ratio on the SOC resistance to decomposition. Mean annual precipitation and soil particle-size distribution also regulated SOC stability indirectly by influencing soil porosity through plant cover and

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biocrust community structure. These findings suggest that proper grazing might not increase the CO_2 release potential or adversely affect SOCS in the biocrust layer. This research provides some guidance for proper grazing management in the sustainable utilization of grassland resources and C sequestration in biocrusts in the hilly regions of drylands.

Keywords: biological soil crusts; livestock grazing; soil organic carbon; biocrust community structure; soil carbon-to-nitrogen ratio; dryland ecosystems; Loess Plateau

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1 Introduction

Hyper-arid, arid, semi-arid and dry sub-humid ecosystems (drylands) are important for the terrestrial carbon (C) cycle, occupying approximately 45.0% of the terrestrial surface and accounting for over 25.0% of the Earth's soil organic carbon (SOC) reserves (Maestre et al., 2013; Prăvălie, 2016). Accordingly, soils of drylands play a major role in stabilizing atmospheric carbon dioxide (CO_2) concentrations (Ahlström et al., 2015). In dryland ecosystems with sparse vascular plants, biological soil crusts (biocrusts) are the dominant living cover (up to 70.0% of the ground surface) and important modulators of the C cycle (Maestre et al., 2013), which are composed of mosses, lichens, cyanobacteria, fungi and bacteria, and other microorganisms (Belnap et al., 2016). As reported, biocrusts are an important source of SOC, which can fix approximately 2.4 Pg of C from the atmosphere every year globally (Elbert et al., 2012), and enrich organic C in the topsoil (biocrust layer) (Gao et al., 2017). Moreover, they largely and complexly regulate the dynamics of soil CO_2 efflux (Yao et al., 2020). Briefly, biocrusts are considered a crucial determinant of soils as a potential sink for the stabilization of atmospheric CO_2 concentrations in drylands. Quantifying the stability of SOC in the biocrust layer is important for understanding the potential of soil to release CO_2 and the role of biocrusts in the C balance of dryland ecosystems.

SOC stability, reflecting the tendency of organic C in soils to resist change and/or loss (Doetterl et al., 2016), is an important indicator of soil C sequestration and mineralization potential. It is considered closely negatively related to the amounts of active C fractions (Blair et al., 1995). It was confirmed that the lability and mineralization potential of SOC in the biocrust layer were remarkably higher than those in the subsurface layers (Baumann et al., 2021). Additionally, the degrees of the biocrust effects on active SOC fractions and SOC stability are closely correlated with crust types and succession stages. With biocrust succession, the communities have stronger C fixation ability (Belnap, 2003) and secrete more amounts and a greater diversity of metabolites, leading to higher labile-C content. It was found that SOC content, labile-C content and its amount relative to the SOC of the biocrust layer in mid- to late-successional biocrusts dominated by mosses or lichens were 2.4–2.9, 2.9–6.8 and 1.2–2.3 times higher than those in early successional biocrusts dominated by cyanobacteria, respectively (Miralles et al., 2013; Gao et al., 2017). Moreover, biocrust succession affects other soil properties that are important for C cycling and turnover. It is well known that biocrust succession positively influences soil aggregation (Yang et al., 2022a), the number of meso-macropores (the meso-macroporosity increased from 3.5%–4.2% in incipient biocrusts to approximately 23.6% in biocrusts dominated by lichens) (Miralles-Mellado et al., 2011), microbial biomass and community diversity (Miralles et al., 2020), and enzymatic activities (e.g., β-glucosidase, cellulose and urease activities) involved in C and nitrogen (N) cycling (Miralles et al., 2012; Zhang et al., 2022). These soil properties, in turn, regulate SOC stability by affecting the accessibility of SOC to microbes (Sollins et al., 1996). Thus, changes in biocrust attributes, such as their successional stages, compositions and cover, may drive the variations in the labile-C content and SOC stability in the biocrust layer and ultimately affect the C balance in biocrust-dominated drylands.

Livestock grazing is the most extensive use of grasslands and a common disturbance source to

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biocrust communities in drylands worldwide (Wu et al., 2020; Maestre et al., 2022). Grazing effects on SOC and biocrust attributes have been a global concern (Schuman et al., 2001; Concostrina-Zubiri et al., 2014; Ferrenberg et al., 2015; Abdalla et al., 2018). Grazing significantly reduces the total biocrust cover (Ferrenberg et al., 2015), and promotes a shift in the biocrust community structure to an early successional state (Bates et al., 2010; Concostrina-Zubiri et al., 2017). In addition, livestock trampling compacts soils and destroys the soil aggregate structure, resulting in a decrease in soil porosity (Faist et al., 2017), which can not only affect the soil humidity and temperature of the biocrust layer (Concostrina-Zubiri et al., 2017; Wu et al., 2023) but also influence the activity of microbes (Bao et al., 2022). Until now, many researchers have explored the grazing effects on SOC without considering the biocrust layer (e.g., Schuman et al., 2001; Abdalla et al., 2018), while only a few have focused on the responses of CO_2 exchange, and SOC sequestration to livestock trampling in biocrust-dominated drylands. It was found that there were different responses of soil CO_2 efflux and SOC to a brief period of simulated grazing disturbance and long-term grazing in different types of soils (Thomas, 2012; Zhang et al., 2016; Yang et al., 2020; Wu et al., 2023). However, it is poorly understood whether and how grazing affects SOC stability in the biocrust layer. The lack of research limits the evaluation of the soil C balance in biocrust-dominated drylands where livestock grazing is widespread.

On the Loess Plateau of China, with the implementation of a large-scale grazing withdrawal program for decades, a large area of grasslands and biocrusts are restored naturally, with total biocrust cover as high as 70.0% (Zhao et al., 2014). Free grazing of livestock in the restored grasslands around rural settlements has been widespread for approximately 10 years. However, in this area, the effects of long-term grazing (for approximately 10 years) on biocrust attributes and functions have been neglected. In particular, the effects of long-term grazing on SOC stability and stock in the biocrust layer and the mechanisms have not yet been fully studied. Therefore, the Loess Plateau is an ideal place to explore the grazing effects on SOC stability in biocrusts. The objectives of this study were to: (1) analyze how long-term grazing impacts biocrust attributes (cover, community structure, biomass and thickness) and soil physical-chemical properties (soil porosity and soil carbon-to-nitrogen ratio (C/N ratio)) in the biocrust layer; (2) evaluate whether and how long-term grazing with different intensities affects SOC stability and stock in the biocrust layer; and (3) determine the mechanisms by which long-term grazing affects SOC stability in the biocrust layer under the influences of environmental conditions (mean annual precipitation (MAP), soil particle-size distribution, and plant cover). We hypothesized that (1) grazing would decrease biocrust cover, biocrust biomass, soil porosity and soil C/N ratio, and alter biocrust community structure; (2) grazing would reduce the SOC stock (SOCS) in the biocrust layer; and (3) grazing and environmental factors would regulate SOC stability by affecting biocrust attributes (cover, community structure and biomass) and soil physical-chemical properties (soil porosity and soil C/N ratio) in the biocrust layer. The results of this study are expected to provide baseline information to quantify the soil C balance in biocrust-dominated drylands and some guidance for the sustainable utilization of grassland resources and SOC sequestration in biocrusts on the Loess Plateau and similar regions in drylands of the world.

2 Materials and methods

2.1 Study area

On the Loess Plateau of China, a large-scale grazing withdrawal program has been launched since 2001 to reverse the grassland degradation trend and promote grassland productivity. As a result, vegetation cover and the quantity and quality of forage were improved markedly in the restored grasslands. However, in the last decade, as the cost of living and production increased for local residents, livestock grazing in the restored grasslands has come up again.

Four independent and typical regions with similar histories of grassland restoration and grazing in the restored grasslands located on the Loess Plateau (Fig. 1) were selected along precipitation

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gradient, namely Dingbian County (37°35′N, 107°35′E; 1303–1907 m a.s.l.), Shenmu City (38°50′N, 110°29′E; 739–1449 m a.s.l.) and Ansai District (36°51′N, 109°19′E; 1012–1731 m a.s.l.) in Shaanxi Province, and Guyuan City (36°00′N, 106°17′E; 1675–2148 m a.s.l.) in Ningxia Hui Autonomous Region. The mean annual precipitation (MAP) varied between 374 and 571 mm over the 2001 to 2020 period, of which 60.0% or more falls between June and September. The mean annual temperature (MAT) ranged from 7.8°C to 9.9°C during the 2001 to 2020 period. The MAP and MAT data were acquired from the National Meteorological Information Centre of the China Meteorological Administration (https://data.cma.cn/). Moreover, the ratio of soil clay and silt content to sand content, representing the soil particle-size distribution, ranged from approximately 0.38 to 0.95 in the four regions (Table 1). All four regions have a consistent topography, and are representatives of hilly or undulating regions in semi-arid areas. In all four regions, goats are the most common livestock. Generally, goats walk and forage freely driven by the herder in summer and autumn, while they are mainly fed with crop gains, crop residue leftovers or other feed supplements during winter and early spring periods. The share of heavily grazed grasslands is lower due to the restriction of grazing withdrawal program.

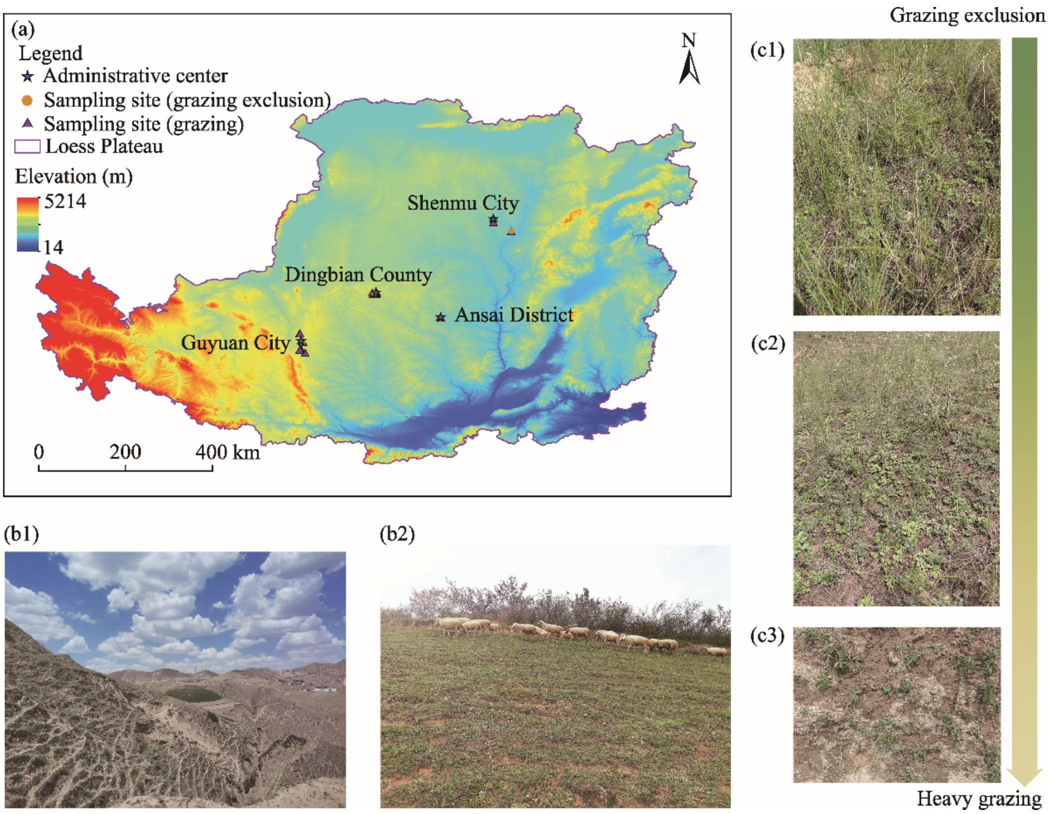

Fig. 1 Overview of the Loess Plateau and distribution of sampling sites on the Loess Plateau (a), the typical landscape of grazing in the hilly regions (b1 and b2), and the land surface characteristics of grasslands along grazing intensity gradient (from grazing exclusion to heavy grazing) (c1–c3).

In all four regions, biocrusts are widely distributed and dominated by cyanobacteria and mosses. The common cyanobacteria species include *Tolypothrix metamorpha*, *Microcoleus vaginatus*, *Phormidium tenue*, *Phormidium calciola* and *Nostoc* spp., while moss species are dominated by *Didymodon tectorum*, *Didymodon vinealis* and *Bryum argenteum*. Lichens are less common, and their cover seldom reaches 10.00% (Zhao et al., 2014). The main soil types include Regosols and Umbrisols, according to the world reference base for soil resources (International Union of Soil Sciences Working Group World Reference Base, 2015).

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Table 1 Variations in meteorological condition and soil particle-size distribution in the biocrust layer in the four regions

Variable	Dingbian County	Shenmu City	Guyuan City	Ansai District
MAP (mm)	374	427	468	571
MAT (°C)	8.0	9.7	7.8	9.9
Soil type (IUSS Working Group WRB, 2015)	Regosols	Regosols	Regosols and Umbrisols	Regosols
Clay content (≤0.002 mm) (%)	12.5±0.4^{b}	10.4±0.8^{c}	18.5±0.9^{a}	14.3±0.8^{b}
Silt content (0.002–0.050 mm) (%)	20.3±0.6^{c}	16.0±1.5^{d}	29.7±0.7^{a}	24.7±1.1^{b}
Sand content (0.050–2.000 mm) (%)	67.2±0.9^{b}	73.6±2.3^{a}	51.8±1.5^{d}	61.0±1.8^{c}
Soil particle-size distribution	0.49±0.02^{c}	0.38±0.04^{d}	0.95±0.06^{a}	0.65±0.05^{b}

Note: MAP, mean annual precipitation (over the 2001 to 2020 period); MAT, mean annual temperature (over the 2001 to 2020 period); IUSS Working Group WRB, International Union of Soil Sciences Working Group World Reference Base; Soil particle-size distribution, the ratio of soil clay and silt content to sand content. Values are means±SD. Different lowercase letters within the same row indicate significant differences among the different regions at $P<0.05$ level.

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2.2 Field survey and sampling

From June to September 2020 (the rainy season), according to the data on livestock on hand in the four regions from the China Statistical Yearbook (county-level) (National Bureau of Statistics of China, 2019) and the current situation of livestock production, we investigated approximately 65 grassland sites with the number of goat dung per square meter as a representation of grazing intensity (Ding and Eldridge, 2020; Freitag et al., 2021) and the frequency of goats entering the grasslands as a reference reported by nearby rural residents. Laing et al. (2003) confirmed that there was a strong positive correlation between grazing intensity and the amount of livestock dung per unit area. The number of goat dung per unit area was measured by the goat dung counts in four to six 5 m×5 m quadrats at each grassland site (Ding and Eldridge, 2020). Finally, 42 representative grassland sites were identified as the sampling sites in this study, in which the number of goat dung per square meter ranged from the lowest to the highest as much as possible, while some of the grassland sites with similar grazing intensity were removed. Limited by the restriction of grazing withdrawal program, of all 42 sampling sites, only 2 sites had a grazing intensity that exceeded 30.00 goat dung/m^2 (39.28 and 73.20 goat dung/m^2, respectively). However, to fit the full range of the grazing intensities presented on the Loess Plateau to the greatest extent, these 2 sites were not removed after serious consideration. We also sampled 3 sites that were strictly excluded from grazing as a control in each region. In view of the grasslands excluded from grazing over 30 years, the 3 sampling sites of grazing exclusion in Guyuan City were completely covered by plant basal and litter without biocrusts, for which the final total sample size was decreased to 51 sites. Specifically, there were 11 sites in Guyuan City, 16 sites in Dingbian County, 15 sites in Shenmu City and 9 sites in Ansai District (Table 2). Each sampling site was over 1 hm^2 and located at least 3 km from all other sampling sites.

A 25 cm×25 cm quadrat frame was used to survey the total biocrust cover (%) and the cover of the two major and visible components of the biocrust community (cyanobacteria and mosses) (Belnap and Phillips, 2001). Each quadrat frame was divided into 25 grids (5 cm×5 cm) to survey the presence and frequency of visible components (cyanobacteria, mosses and lichens) in the grids and to assess the cover of the biocrust community in the quadrat frame. Survey quadrat frames were placed in 25–35 random locations within each sampling site. Biocrust community structure was assessed as the relative cover of the above two major components of the biocrust community (Ferrenberg et al., 2015). The relative cover of cyanobacteria (mosses) (%) was calculated by the ratio of cyanobacteria cover (moss cover) to the total biocrust cover. Biocrust thickness (mm) was measured by a vernier caliper. In addition, 6 random quadrats (1 m×1 m) were used to investigate the main plant species, plant cover (%) and litter cover (%) at each sampling site (Ding and Eldridge, 2020). The altitude (m) and slope gradient (°) were recorded respectively at each sampling site.

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Table 2 Characteristics of sampling sites (under grazing and grazing exclusion) in the four regions

Region	Number of sampling sites	Number of goat dung per square meter (goat dung/m^2)	Elevation(m)	Slope gradient (°)	Main plant species	Plant cover (%)	Litter cover (%)
Dingbian County	16						
Grazing exclusion	3	0.00	1657–1662	10–15	*Stipa bungeana*, *Stipa capillata* and *Potentilla reptans*	35.0–55.0	35.8–41.1
Grazing	13	4.15–29.97	1526–1701	15–30	*Thymus mongolicus*, *S. bungeana*, *Oxytropis racemosa* and *Artemisia sacrorum*	16.3–61.7	14.3–41.0
Shenmu City	15						
Grazing exclusion	3	0.00	1209–1214	5	*S. bungeana*, *Lespedeza bicolor* and *Poa sphondylodes*	32.0–63.0	21.2–37.4
Grazing	12	5.03–73.20	1192–1522	10–20	*Artemisia desertorum*, *S. bungeana*, *L. bicolor* and *P. sphondylodes*	5.0–54.0	2.4–43.1
Guyuan City	11						
Grazing exclusion	0	0.00	1952–2079	6–11	*Carex dahurica* and *A. sacrorum*	90.0–95.0	5.0–10.0
Grazing	11	0.88–26.16	1675–2010	5–25	*S. bungeana*, *A. sacrorum*, *P. reptans*, *Potentilla tanacetifolia* and *T. mongolicus*	41.7–78.3	8.6–37.9
Ansai District	9						
Grazing exclusion	3	0.00	1199–1215	18–23	*A. sacrorum*	48.3–56.7	13.2–20.4
Grazing	6	0.28–19.18	1178–1208	10–19	*Bothriochloa ischcemum*, *L. bicolor* and *S. bungeana*	31.7–86.0	14.3–36.4

After the above surveys, 8 samples (size of 8 cm×8 cm) of the biocrust layer (approximately 4.00–13.00 mm thick) at each sampling site were randomly collected using a spatula, and the sampling points were kept away from grass tussocks to avoid potential interference from plant roots. Then, the 8 samples were homogenized to provide one composite sample. Meanwhile, 6 random undisturbed samples of the biocrust layer were collected at each sampling site using Petri dishes. All the samples were dried and then sent to the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau in China. In the laboratory, after biocrustal organism tissues and other impurities were removed, the composite samples were sieved to 1.00 and 0.25 mm for the following laboratory analysis.

2.3 Laboratory analysis

The undisturbed samples were used to determine the soil bulk density (g/cm^3) of the biocrust layer, and biocrust biomass (moss biomass (g/dm^2) and cyanobacterial biomass (mg/m^2)). The soil bulk density of the biocrust layer was determined using the wax-coated and water immersion method (Brasher et al., 1966). Soil porosity (%) was calculated from the soil particle density (which was taken as 2.65 g/cm^3) and soil bulk density through the following empirical equation (Mondal and Chakraborty, 2022):

$$\text{Soil porosity} = (1 - \text{soil bulk density} / \text{soil particle density}) \times 100\%. \quad (1)$$

Moss biomass was estimated using the dry weight of the mosses per unit area (g/dm^2), as described by Gao et al. (2017). Cyanobacterial biomass was represented by the chlorophyll *a* content per unit soil surface area (mg/m^2). Chlorophyll *a* was double extracted with ethanol and measured on a spectrophotometer at a wavelength of 665 nm (Castle et al., 2011). The mosses were completely removed when preparing the cyanobacteria crust samples.

The composite samples were used to determine the soil particle-size distribution, SOC content, soil total N content and SOC fractions of different lability contents. Soil particle-size distribution was determined using the laser-diffraction method (Mastersizer 2000 Laser Diffraction Particle Analyzer, Malvern Instruments Ltd., Worcestershire, UK), as described by Gao et al. (2017). The SOC content (g/kg) was determined by the Walkley Black method (Nelson and Sommers, 1983).

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The total N content (g/kg) was determined using the Kjeldahl method (Bremner and Mulvaney, 1983). The soil C/N ratio was calculated as the ratio of SOC content to soil total N content.

The SOC fractions of different lability contents were measured by potassium permanganate ($KMnO_4$) oxidation, as described by Loginow et al. (1987) and Lefroy et al. (1993). The change in the concentration of $KMnO_4$ was used to estimate the content of C oxidized, assuming that 1.00 mmol/L of MnO_4^- was consumed ($Mn^{7+} \rightarrow Mn^{2+}$) in the oxidation of 0.75 mmol/L (9.00 mg) of C. Four C fractions of different lability contents were measured by treating a soil sample via three different concentrations of potassium permanganate. Specifically, C1 represents the very labile C (g/kg), which is oxidized by 33.00 mmol/L $KMnO_4$; C2 represents the labile C (g/kg), which is oxidized by 167.00 mmol/L $KMnO_4$ but not oxidized by 33.00 mmol/L $KMnO_4$; C3 represents the less labile C (g/kg), which is oxidized by 333.00 mmol/L $KMnO_4$ but not oxidized by 167.00 mmol/L $KMnO_4$; and C4 represents the nonlabile C (g/kg), which is not oxidized by 333.00 mmol/L $KMnO_4$.

For decades, it has become a reliable method to determine the stability of SOC using the ratio of passive C fraction content to active C fraction content (Blair et al., 1995). Specifically, Chan et al. (2001) defined C1 and C2 as active C fractions while C3 and C4 as passive C fractions. In this study, the SOC stability index (SI) was calculated as follows:

$$\mathrm{SI} = \mathrm{C_{passive}\ content}/\mathrm{C_{active}\ content} = (\mathrm{C3\ content} + \mathrm{C4\ content})/(\mathrm{C1\ content} + \mathrm{C2\ content}), \quad (2)$$

where $C_{passive}$ is the passive C fraction (g/kg) and C_{active} is the active C fraction (g/kg).

The SOCS of the biocrust layer (Mg/hm^2) was calculated using SOC content, soil bulk density, biocrust thickness and total biocrust cover as follows:

$$\mathrm{SOCS} = \mathrm{SOC\ content} \times \mathrm{soil\ bulk\ density} \times \mathrm{biocrust\ thickness} \times \mathrm{total\ biocrust\ cover} \times 0.01. \quad (3)$$

2.4 Statistical analysis

Linear and nonlinear (exponential) regression analyses were used to explore the changes in SOC content and SOC fractions of different lability contents, SI, SOCS, biocrust attributes and soil physical-chemical properties with increasing grazing intensity. In addition, we divided grazing intensity into four grades according to the number of goat dung per square meter: 0.00, 0.00–10.00, 10.00–20.00, and ≥20.00 goat dung/m^2. The SOCS data were first tested for normality using the Kolmogorov-Smirnov test and for equality with the Levene's test, of which the significance of the differences among grazing intensity grades was analyzed using one-way analysis of variance (ANOVA) and the least significant difference. Meanwhile, to reduce the influence of environmental conditions on SOCS in the four regions, we performed a multi-way covariance (ANCOVA) with grazing intensity grade as a fixed factor and the environmental factors (MAP, MAT, altitude, plant cover, litter cover and soil particle-size distribution) as covariates. The above analyses were performed using SPSS 25.0 statistical software (SPSS Inc., Chicago, USA). The figures were generated using Origin 2022 (Origin Lab Corp., Northampton, USA).

A structural equation model (SEM) was applied using Amos 21.0 (SPSS Inc., Chicago, USA) to explore how grazing affected SOC stability in the biocrust layer under different environmental conditions. In this study, we hypothesized that grazing intensity, MAP, soil particle-size distribution and plant cover in each region have direct or indirect effects (via biocrust attributes, soil porosity and soil C/N ratio) on SOC stability. To determine a biocrust attribute indicator representing the effect of grazing on SOC stability in the biocrust layer under different environmental conditions, we used Pearson correlation analysis to identify which biocrust attribute (e.g., cover, community structure, biomass and thickness) was most strongly correlated with SI, MAP, soil particle-size distribution, plant cover, soil porosity and soil C/N ratio. Once the model was constructed, we used the maximum likelihood estimation technique to parameterize the model and conducted goodness-of-fit test (chi-square test; Joreskog's goodness-of-fit index (GFI)). In the chi-square test, a high *P* value is desired ($P>0.05$). Other fit indices are interpreted using rules-of-thumb; specifically, $\chi^2/df<2.000$, root mean square error of approximation (RMSEA)<0.080 and Joreskog's GFI>0.950 are considered to indicate a good fit (Grace, 2006).

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3 Results

3.1 Variations in biocrust attributes and soil physical-chemical properties of the biocrust layer with grazing intensity

Figures 2 and S1 show the grazing effect on biocrust attributes and soil physical-chemical properties in the biocrust layer. It was found that grazing did not significantly change total biocrust cover (P=0.441; Fig. S1a) but altered cyanobacteria cover and moss cover (Fig. 2a and d). With increasing grazing intensity, cyanobacteria cover increased with nonsignificant trend (P=0.069); in contrast, moss cover decreased significantly and linearly (P=0.014). For biocrust community structure, on average, the relative cover of cyanobacteria increased from 64.9% under grazing exclusion to 79.9% under grazing (Fig. 2c). Mosses comprised 20.0% of the cover in the biocrust community under grazing, compared with 34.6% of the cover in the biocrust community under grazing exclusion (Fig. 2f). That was to say, compared with that under grazing exclusion, the mean relative cover of cyanobacteria under grazing increased by 23.1%, while the mean relative cover of mosses decreased by 42.2%. Similar to the changes in the biocrust community structure, the mean cyanobacterial biomass increased by 8.8% and the mean moss biomass decreased by 33.4% under grazing, compared with those under grazing exclusion (Fig. 2b and e). The mean biocrust thickness under grazing was 14.2% lower than that under grazing exclusion (Fig. 2g).

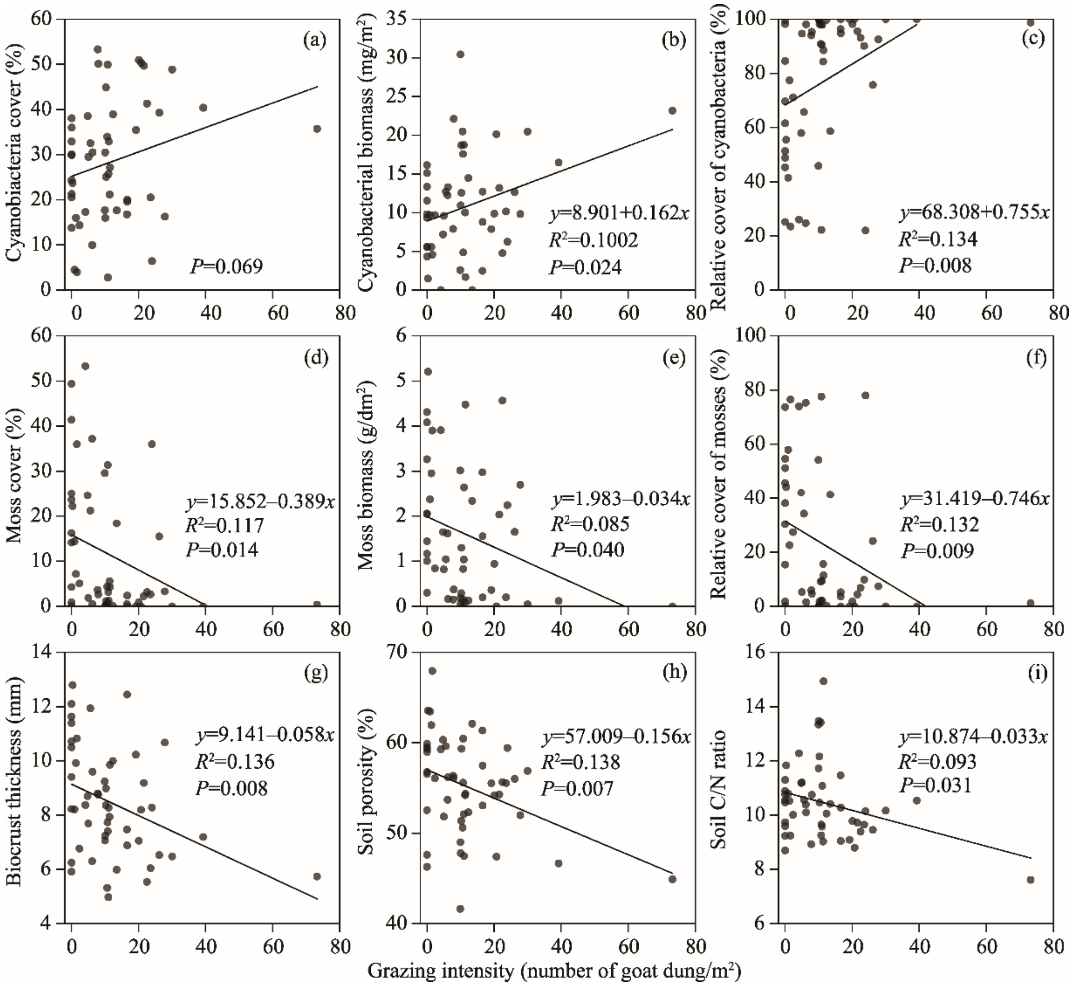

Fig. 2 Variations in biocrust attributes of cover (a and d), biomass (b and e), relative cover (c and f) and thickness (g), as well as soil physical-chemical properties of soil porosity (h) and soil C/N ratio (i) in the biocrust layer with increasing grazing intensity. Soil C/N ratio, soil carbon-to-nitrogen ratio. The linear or nonlinear regression equations are shown at P<0.05 level but omitted at P>0.05 level. The same below.

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The soil particle-size distribution was not significantly related to grazing (P=0.926; Fig. S1c). The soil porosity (P=0.007) and soil C/N ratio (P=0.031) in the biocrust layer significantly decreased by 4.1%–7.2% and 7.2%–13.3% under grazing, respectively, compared with those under grazing exclusion (Fig. 2h and i).

3.2 Variations in the SOC fractions of different lability contents and SOC stability index (SI) of the biocrust layer with grazing intensity

Results of the variations in the SOC content, SOC fractions of different lability contents and SI in the biocrust layer with increasing grazing intensity are indicated in Figure 3. Overall, the SOC content in the biocrust layer ranged between 4.43 and 26.16 g/kg and decreased exponentially with increasing grazing intensity (P=0.007; Fig. 3a). Compared with that under grazing exclusion, the SOC content under grazing was reduced by 1.2%–26.2%. For the SOC fractions of different lability contents, the C1 content decreased exponentially by 0.0%–25.6% (P=0.014; Fig. 3b) and the C4 content decreased significantly by 4.3%–27.8% (P=0.045; Fig. 3e) under grazing, compared with those under grazing exclusion. However, no significant decreases were observed in the C2 content (P=0.150; Fig. 3c) and C3 content (P=0.570; Fig. 3d). In addition, significant variations were not found in the relative contents of C1 (P=0.470), C2 (P=0.630), C3 (P=0.526) and C4 (P=0.469) to SOC (Fig. S2). On average, the relative contents of C1, C2, C3 and C4 to SOC were 9.6%, 9.2%, 10.1% and 71.1%, respectively. Long-term grazing had no significant effect on SI in the biocrust layer. The average SI was 4.24 under grazing and 4.52 under grazing exclusion (Fig. 3f).

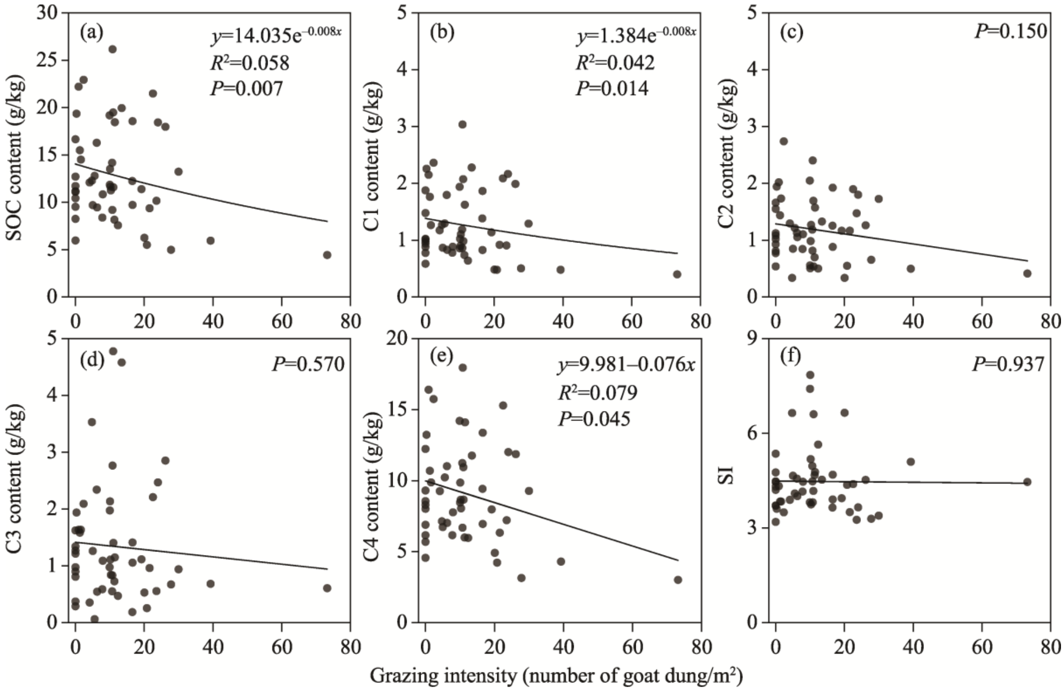

Fig. 3 Variations in the SOC content (a), SOC fractions of different lability contents (b–e) and SI (f) in the biocrust layer with increasing grazing intensity. SOC, soil organic carbon; C1, very labile C; C2, labile C; C3, less labile C; C4, nonlabile C; SI, SOC stability index.

3.3 Effect of grazing on SOCS in the biocrust layer

A significant increase was found for the soil bulk density in the biocrust layer with increasing grazing intensity (P=0.007; Fig. S1b). Moreover, according to the changes in SOC content and biocrust cover and thickness, the SOCS decreased significantly and linearly with increasing

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grazing intensity (P=0.020) and ranged from 0.13 to 1.30 Mg/hm^2 (Fig. 4a). The mean SOCS under grazing (0.49 Mg/hm^2) was 15.5% lower than that under grazing exclusion (0.58 Mg/hm^2).

After grading the grazing intensity by classifying the number of goat dung per unit area, we found that there was no statistically significant difference between the SOCS of the biocrust layer under the grazing intensity of 0.00–10.00 goat dung/m^2 (mean value of 0.61 Mg/hm^2) and that under grazing exclusion (mean value of 0.58 Mg/hm^2) (Fig. 4b). The SOCS under the grazing intensity of more than 10.00 goat dung/m^2 (mean value of 0.41 Mg/hm^2) was not significantly different from that under grazing exclusion but was significantly lower than that under the grazing intensity of 0.00–10.00 goat dung/m^2. Furthermore, the results of the multi-way ANCOVA indicated that grazing intensity and other environmental factors (including MAP, MAT, litter cover and soil particle-size distribution) had no significant effect on SOCS, while altitude and plant cover had significant effects on SOCS in the biocrust layer (P=0.008 and P=0.036, respectively; Table 3).

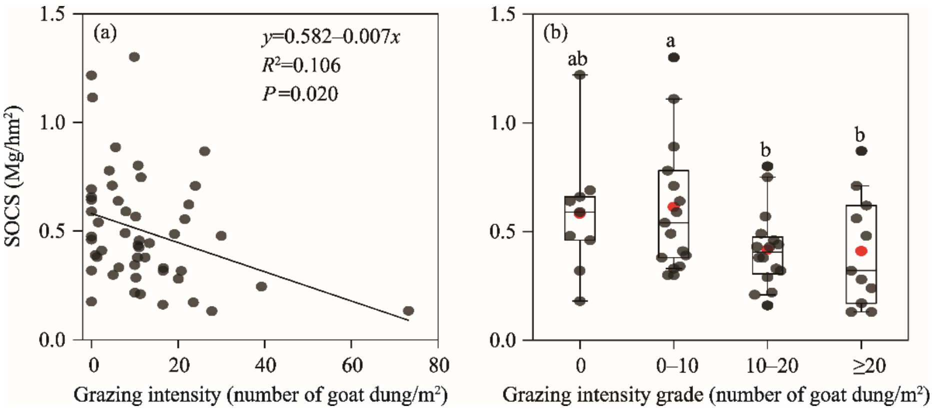

Fig. 4 Variations in SOC stock (SOCS) in the biocrust layer with increasing grazing intensity (a) and under different grazing intensity grades (b). Different lowercase letters indicate significant differences among different grazing intensity grades (P<0.05). The lower and upper boundaries of the box indicate the 25th and 75th percentiles, respectively; whiskers below and above the box indicate the 10th and 90th percentiles, respectively. The black horizontal line within each box indicates the median value, and the red dot indicates the mean value.

Table 3 Results of the multi-way analysis of covariance (ANCOVA) showing the impacts of environmental conditions and grazing intensity grades on SOC stock (SOCS) in the biocrust layer

Impact factor	Mean square	F	P
MAP	0.105	1.963	0.169
MAT	0.123	2.314	0.136
Altitude	0.408	7.657	0.008**
Plant cover	0.252	4.715	0.036*
Litter cover	0.171	3.208	0.081
Soil particle-size distribution	0.001	0.024	0.876
Grazing intensity grades	0.083	1.553	0.215

Note: * indicates significance at P<0.05 level, and ** indicates significance at P<0.01 level.

3.4 Influence mechanism of grazing on SOC stability in the biocrust layer

SI and soil porosity were more strongly correlated with the relative cover of cyanobacteria and the relative cover of mosses than with total biocrust cover, cyanobacteria cover and biomass, moss cover and biomass, and biocrust thickness (Table 4). Moreover, the relative cover of cyanobacteria and the relative cover of mosses were significantly related to plant cover and soil particle-size distribution. All the biocrust attributes had no significant relationship with MAP or soil C/N ratio. Consequently, the relative cover of cyanobacteria was used to represent the biocrust attributes under grazing and environmental conditions.

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Table 4 Pearson correlation coefficients of biocrust attributes with grazing intensity, environmental conditions, soil physical-chemical properties and SI

Variable	Total biocrust cover	Cyanobacteria cover	Moss cover	Cyanobacteria biomass	Moss biomass	Relative cover of cyanobacteria	Relative cover of mosses	Biocrust thickness
Grazing intensity	–0.138	0.256	–0.342*	0.317*	–0.289*	0.366**	–0.363**	–0.369**
MAP	0.043	–0.237	0.240	–0.204	0.023	–0.248	0.242	0.135
Plant cover	0.006	–0.448**	0.376**	–0.296*	0.231	–0.482**	0.479**	0.018
Soil particle-size distribution	0.015	–0.253	0.200	–0.155	0.081	–0.298*	0.300*	–0.105
Soil porosity	0.130	–0.367**	0.445**	–0.451**	0.429**	–0.524**	0.524**	0.355*
Soil C/N ratio	–0.100	–0.140	0.047	–0.177	0.227	–0.023	0.025	–0.014
SI	–0.035	0.236	–0.234	0.238	–0.239	0.277*	–0.276*	–0.173

Note: Soil C/N ratio, soil carbon-to-nitrogen ratio; SI, SOC stability index. * indicates significant differences at P<0.05 level, and ** indicates significant differences at P<0.01 level.

To reveal the mechanism by which grazing influenced SOC stability in the biocrust layer under different environmental conditions, we constructed an SEM to determine how grazing regulated SOC stability by affecting biocrust attributes and soil physical-chemical properties in the biocrust layer (Fig. 5). The final model explained 25% of the variance in SOC stability and fitted the data well (χ^2/df=1.106, P=0.356, R^2=0.250, GFI=0.963 and RMSEA=0.046). The SEM results showed that grazing intensity, MAP and soil particle-size distribution indirectly affected SOC stability in the biocrust layer by affecting soil porosity and soil C/N ratio. Above all, grazing directly decreased soil porosity (standardized path coefficient (β)= –0.26 and P=0.027) and soil C/N ratio (β= –0.35 and P=0.010), and indirectly decreased soil porosity by increasing the relative cover of cyanobacteria caused by decreased plant cover. In addition, MAP and soil particle-size distribution in each region also affected soil porosity directly and indirectly by influencing plant cover and then regulating the relative cover of cyanobacteria. Finally, soil porosity (β= –0.38 and

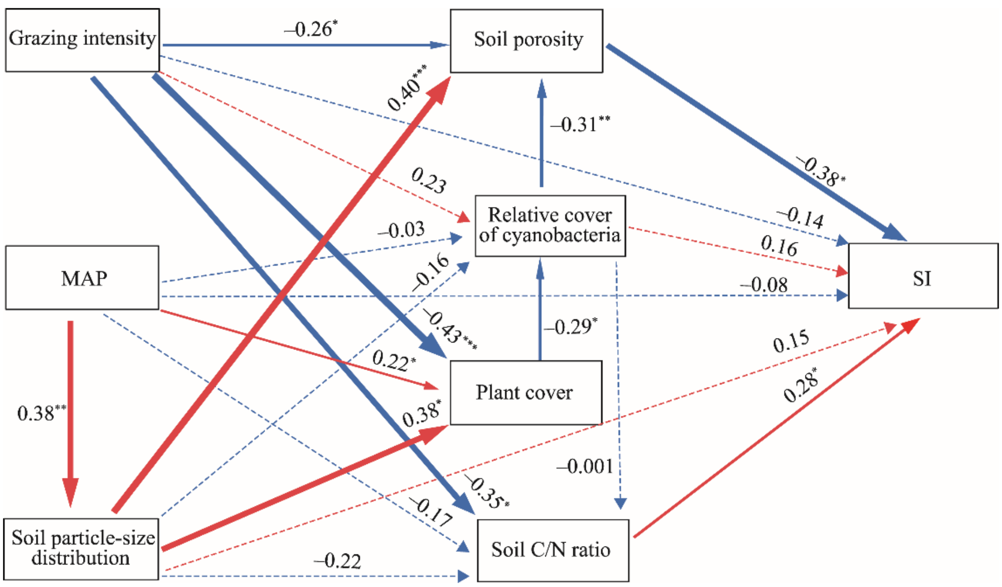

Fig. 5 Structural equation model (SEM) showing the mechanisms by which grazing affected SOC stability in the biocrust layer under the influences of environmental factors. The arrow direction represents hypothetical causality. Standardized path coefficient (β) close to the arrow indicates the effect size of the relationship. The red and blue arrows indicate positive and negative relationships, respectively. The width of the arrows is proportional to the coefficient. Significant coefficients are represented by values with asterisks, while nonsignificant coefficients (P>0.05) are represented by values without asterisks. *, P<0.05 level; **, P<0.01 level; ***, P<0.001 level.

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P=0.025) and soil C/N ratio (β=0.28 and P=0.049) directly negatively and positively influenced SOC stability, respectively. The results implied that the decrease in soil porosity caused by grazing, either directly or indirectly, could promote SOC stability; instead, the decrease in soil C/N ratio caused by grazing could reduce SOC stability.

4 Discussion

4.1 Long-term grazing effects on SOCS in the biocrust layer

Biocrusts occupy a large part of the soil surface in dryland ecosystems and play multiple critical ecological roles in ecosystem functions (Belnap et al., 2016), especially in favor of the accumulation and stabilization of organic C (Sancho et al., 2016). Livestock grazing is a common disturbance source to biocrust communities in drylands worldwide and becomes an important factor affecting SOC stability and the C balance of dryland ecosystems (Thomas, 2012; Zhang et al., 2016; Yang et al., 2020; Wu et al., 2023). Inconsistent with our hypothesis, the total biocrust cover under grazing did not decrease as significantly as that in previous studies (Ding and Eldridge, 2020; Root et al., 2020). The contrasting variation might be related to the common networks of livestock tracks formed by long-term goat trampling on hilly or undulating topography (Jin et al., 2016) (Fig. 1b). Biocrusts are always found in nontrack areas and may be protected as much as possible from goat trampling. Nevertheless, grazing promoted a shift in the biocrust community to an early successional state, with increases in the relative cover of cyanobacteria and declines in the relative cover of mosses (Fig. 2c and f). The shift in biocrust community structure is closely related to the high resistance and resilience of cyanobacteria to grazing and the change in microenvironmental conditions. First, cyanobacteria are more likely to colonize soil surfaces left vacant by the removal of mosses, due to their protective polysaccharide sheaths, their greater ability to move through the soil and be dispersed by wind and water, and their high tolerance to a wide range of environmental conditions (low to high light, ultraviolet, soil temperature and soil moisture) (Concostrina-Zubiri et al., 2014). In addition, grazing induced the reduction in plant cover, which limited the growth and development of mosses (Fig. 5), and promoted a shift in the biocrust community to an early successional state dominated by cyanobacteria (Belnap et al., 2001). The shifted biocrust community structure resulted in a lower C fixation ability, which was further reflected by the reductions of the SOC content and SOCS in the biocrust layer under higher grazing intensity (≥10.00 goat dung/m^2) (Housman et al., 2006; Pietrasiak et al., 2013).

The SOCS represents the net result of long-term changes in SOC gains and losses (Sancho et al., 2016). Thomas (2012) found that the response of SOCS to grazing in biocrusts varied in different types of soils. Largely consistent with the study in the Mu Us Sandy Land of China (Wu et al., 2023), there was no adverse effect of light grazing intensity on SOCS in our study (Fig. 4b), which might be attributed to the slower water evaporation caused by the broken biocrust patches and the longer duration of photosynthetic activity. Additionally, the formation of livestock tracks might result in changes in the hydrological processes, further leading to different patterns in the spatial distribution of water (Jin et al., 2016). However, the mechanism underlying the negative response of SOC balance to intermediate and heavy grazing/disturbance may be different because no sand burial phenomenon was found in our study. Further studies are needed to verify whether grazing worsens soil erosion and affects the SOCS in the biocrust layer through soil C loss from soil erosion in the hilly regions.

The impacts of grazing on ecosystem services strongly depend on the environmental conditions (e.g., climate, soil and other biotic features) (Maestre et al., 2022). In this study, in addition to plant cover, altitude regulated the grazing effects on SOCS in the biocrust layer (Table 3; Fig. S3a). Altitude alters solar radiation, soil temperature and soil moisture, influencing the photosynthesis of biocrusts. Moreover, lower soil temperatures at higher altitudes can suppress soil microorganisms and their activities, which will result in less decomposition of SOC and indirectly promote SOC accumulation (Liu et al., 2009; Xu et al., 2022). MAP indirectly affected

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biocrust attributes and SOCS in the biocrust layer by affecting plant cover (Table 3; Fig. 5), which might be because increased precipitation can promote the growth of plants, especially in drylands (Bowker et al., 2016). Additionally, the soil matrix acted as the vessel of SOC, which modified SOC stability and turnover rates through soil physical structure and chemical processes (Figs. 5 and S3b) (Luo et al., 2017). It could also influence the capacity of soil to retain moisture and fertility, ultimately altering the biocrust community structure by affecting plant growth (Fig. 5) (Ding and Eldridge, 2020). However, the soil particle-size distribution had no significant effect on SOCS in the biocrust layer (Zhou et al., 2019).

4.2 SOC stability in the biocrust layer remained unchanged under long-term grazing

The stability of SOC in the biocrust layer, which is enriched with organic C, is an important factor affecting CO_2 emissions and soil C balance in dryland ecosystems. Livestock excrement can provide easily decomposed C resources (Eldridge and Delgado-Baquerizo, 2018), compensating for the reduction in C1 and C2 contents caused by the shifted biocrust community structure (Fig. S4), thus ensuring a stable ratio of active C fraction content to passive C fraction content under long-term grazing. The ratio of passive C fraction (resistant to decomposition by soil microorganisms) content to active C fraction (easily decomposed) content in the biocrust layer did not change, that is, the potential of soil in biocrusts to release CO_2 remained unchanged. This result disagreed with the study of Yang et al. (2020), who found that short-term trampling disturbance increased the relative content of active organic C to SOC. This difference might be due to the inconsistent disturbance patterns (uniform human disturbance and livestock disturbance) and the different grazing durations (short-term and long-term). There are no networks of livestock tracks to protect biocrusts under short-term trampling disturbance.

The unchanged SOC stability under grazing was regulated by the variations in biocrust attributes and soil physical-chemical properties. As shown in the SEM, grazing balanced SOC stability in the biocrust layer by regulating soil porosity and soil C/N ratio. First, the downward compressional forces from livestock trampling resulted in a higher soil bulk density and breakdown in macroporosity (Belnap, 2006; Faist et al., 2017). In addition, the reverse succession of biocrusts not only reduced soil porosity, but also changed the pores from larger, interconnected elongated and irregular pores to smaller unconnected rounded pores (Miralles-Mellado et al., 2011). Besides, the increase in fine particles of soils could promote the total pore space and the relative number of small pores (Warren, 2001). The decrease in soil porosity and the relative number of large interconnected pores are not conducive to the diffusion of soil gas, which could not only reduce bacterial abundance, but also make it difficult for microorganisms to use oxygen and make SOC inaccessible to microorganisms and enzymes (Sollins et al., 1996; Ramírez et al., 2020), thereby limiting the decomposition of organic matter and enhancing the stability of SOC (Kravchenko and Guber, 2017).

In addition, due to the N resources provided by livestock excrement deposition, soil C/N ratio decreased significantly with increasing grazing intensity (Chen et al., 2021). Soil C/N ratio and the shift in biocrust community structure could directly affect the structure and function of the soil microbial community (Bahram et al., 2018; Miralles et al., 2020). It was confirmed that grazing disturbance could decrease soil fungal functional groups (Eldridge and Delgado-Baquerizo, 2018) and increase the bacterial/fungal biomass ratio (Bao et al., 2022). Xu et al. (2016) found that the decomposition rate of the active and slow SOC pools decreased with increasing soil C/N ratio. Thus, in contrast to the effect of the reduction in soil porosity, grazing induced a lower soil C/N ratio, leading to a change in microbial C use efficiency, ultimately favoring soil respiration and the lability of SOC (Riggs and Hobbie, 2016).

Put simply, the various environmental conditions (climate, topography and soil texture) and grazing durations (long-term and short-term) in different regions might result in different patterns of SOC stability in the biocrust layer.

4.3 Implications for grassland management

In drylands, due to water scarcity, biocrusts occupy a large part of the soil surface and play a key

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role in the soil C cycle, especially in the biocrust layer; likewise, a large proportion of the population relies heavily on livestock grazing for subsistence here (Maestre et al., 2022). However, the effects of grazing on SOC stability and sequestration in the biocrust layer have often been neglected in previous studies (e.g., Schuman et al., 2001; Abdalla et al., 2018). In this study, we focused on biocrusts, which play important roles in ecosystem stability and development, and found that light grazing (intensity of 0.00–10.00 goat dung/m^2) could maintain the SOCS in the biocrust layer. We also found that light grazing promoted the development of vegetation communities, such as increasing the average cover, biomass and diversity (Sun et al., 2022). In addition, for another important biocrust ecological function in the hilly or mountainous regions, it is encouraging that the average biocrust cover under light grazing was 43.2%, more than the threshold at which soil erosion could be well controlled (Yang et al., 2022b). We estimated the grazing intensity (goat/(hm^2·a)) based on the quantitative relationship between grazing intensity and the number of goat dung per unit area determined in our controlled grazing experiments in the fenced grasslands in the same study area (Fig. S5), and revealed that the grazing intensity corresponding to 0.00–10.00 goat dung/m^2 was approximately 1.20–2.60 goat/(hm^2·a). Therefore, it follows that compared with grazing exclusion, light grazing (intensity of approximately 1.20–2.60 goat/(hm^2·a)) could improve local farmers' income without degrading the ecosystem services in the restored grasslands. Our findings provide a key basis for future government policy decisions on the effective management of restored grasslands in grazing withdrawal program on the Loess Plateau of China.

Moreover, it seems reasonable to extend the influence mechanisms by which long-term grazing balanced SOC stability in the biocrust layer to other hilly, undulating or mountainous regions of drylands. It is easy to understand that the formation of livestock tracks is largely biogenic on hilly, undulating or mountainous topography, as using near-horizontal tracks for foraging would be energy efficient for goats (Jin et al., 2016). The protection of the typical networks of livestock tracks may be important in maintaining biocrust ecological functions under grazing in the hilly regions. However, grazing may not have equal effects on C sequestration in biocrusts and ecosystem services in different areas due to regional differences in environmental conditions. For instance, livestock trampling tends to compress the surface of plains more severely, which may result in distinct degrees and directions of changes in SOC stability and stock. Moreover, in most arid and semi-arid desert areas, livestock trampling often turns soils over and buries biocrustal organisms, making biocrusts on sandy soils largely more susceptible to rapid degeneration, and recovery may be slower or even prevented (Thomas, 2012; Wu et al., 2020, 2023). Additionally, it is noteworthy that the interactions among biocrusts and vascular plants are complex under different climate conditions. In humid areas, vascular plants, which dominate the vegetation community, restrict biocrusts to small open patches. Under these conditions, grazing-induced decreases in plant cover may increase biocrust cover (Warren and Eldridge, 2001). In contrast, in arid areas, biocrusts occupy the interspaces between sparse vascular plants due to their high tolerance to desiccation; biocrust attributes and functions may respond negatively to livestock trampling (Belnap, 2006). The effect of grazing on biocrusts in different climate conditions will inevitably lead to different responses of SOCS in the biocrust layer. More research in other regions worldwide could help us better understand the effect of grazing on SOC stability and sequestration in biocrusts and their regulated pathways. There is still much work for researchers to do in determining the optimal grazing intensity, frequency, timing, etc., according to local conditions, without harming biocrust attributes and ecological functions.

Although the estimated grazing intensity may not be exactly consistent with the true grazing intensity in this study, this should not be a major weakness (Laing et al., 2003; Freitag et al., 2021). Additionally, due to the restriction of grazing withdrawal program, the results from only 2 sampling sites with more than 30.00 goat dung/m^2 may not be an accurate representation of SOC stability and stock in the biocrust layer under high grazing intensity; however, the variation direction of SOC stability and stock with grazing intensity was established. Because the sampling sites in this study may still allow a true representation, the area of each sampling site was large

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enough (approximately 1 hm^2) and the conditions (slope and aspect) were assured to be similar among sampling sites. Another important point is that it is of great significance for regional practical guidance to reserve the maximum range of grazing intensity presented on the Loess Plateau.

5 Conclusions

Biocrusts are an important component of dryland ecosystems, enriching the upper millimeters of the soil with organic C. In the context of global climate change, it is very important to determine the responses of SOC stability and stock in the biocrust layer to grazing to quantify the C balance in biocrust-dominated drylands where livestock grazing is widespread. The results showed that long-term grazing did not change SOC stability but indirectly decreased the mean SOCS by 15.5% in the biocrust layer in the hilly regions of drylands. However, light grazing did not adversely affect SOCS. Specifically, long-term grazing altered biocrust community structure and decreased soil porosity and soil C/N ratio, rather than biocrust cover. Grazing regulated SOC stability via variations in biocrust community structure (the relative cover of cyanobacteria), soil porosity and soil C/N ratio under different environmental conditions (MAP, soil particle-size distribution and plant cover). Therefore, proper grazing might not increase the CO_2 release potential or adversely affect SOCS in the biocrust layer in the hilly regions of drylands. These findings provide effective guidance for scientific grazing management in the sustainable utilization of grassland resources and C sequestration in biocrusts in the hilly regions of drylands.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conceptualization: ZHAO Yunge, XU Mingxiang, MA Xinxin; Methodology: MA Xinxin, ZHAO Yunge, YANG Kai; Formal analysis and investigation: MA Xinxin, QIAO Yu, YANG Kai, MING Jiao; Writing - original draft preparation: MA Xinxin, YANG Kai; Writing - review & editing: MA Xinxin, ZHAO Yunge, XU Mingxiang, YANG Kai, MING Jiao, PAN Xinghui; Funding acquisition: ZHAO Yunge, XU Mingxiang; Resources: ZHAO Yunge; Supervision: ZHAO Yunge, XU Mingxiang.

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Appendix

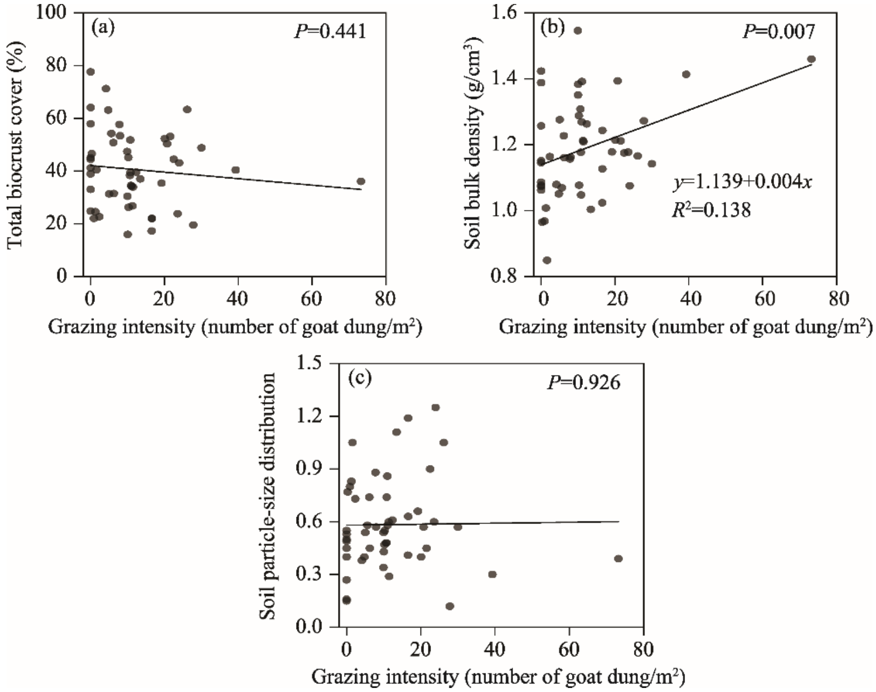

Fig. S1 Variations in total biocrust cover (a), soil bulk density (b) and soil particle-size distribution (c) in the biocrust layer with increasing grazing intensity. Soil particle-size distribution is the ratio of soil clay and silt content to sand content.

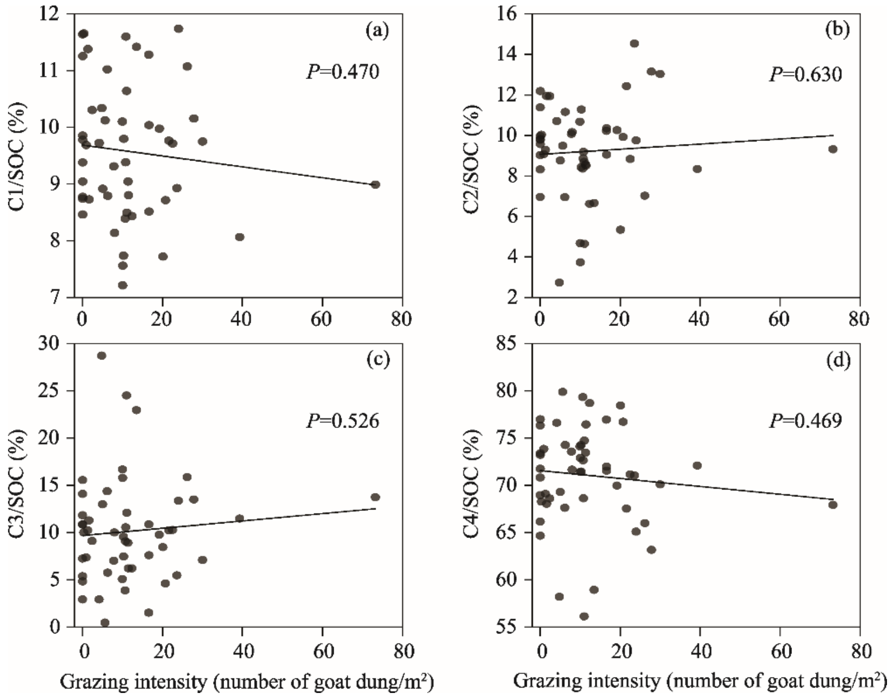

Fig. S2 Variations in the relative contents of C1, C2, C3 and C4 to SOC in the biocrust layer with grazing intensity. (a), C1/SOC; (b), C2/SOC; (c), C3/SOC; (d), C4/SOC. C1, very labile C; C2, labile C; C3, less labile C; C4, nonlabile C.

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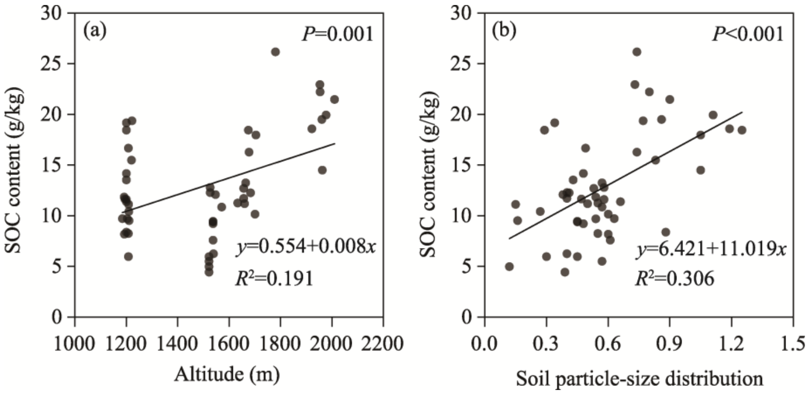

Fig. S3 Relationships of the SOC content of the biocrust layer with altitude (a) and soil particle-size distribution (b)

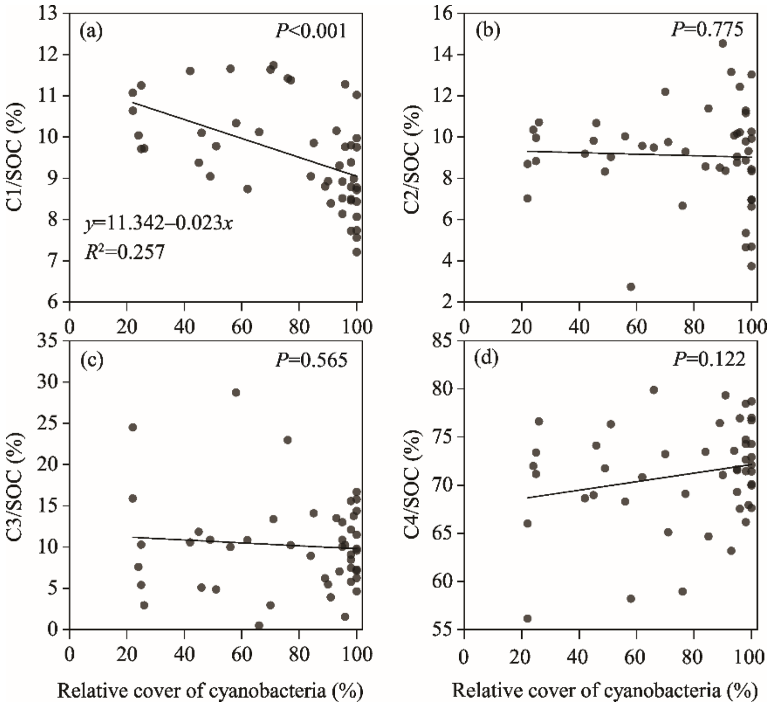

Fig. S4 Relationships of the relative cover of cyanobacteria with the relative contents of C1, C2, C3 and C4 to SOC in the biocrust layer. (a), C1/SOC; (b), C2/SOC; (c), C3/SOC; (d), C4/SOC.

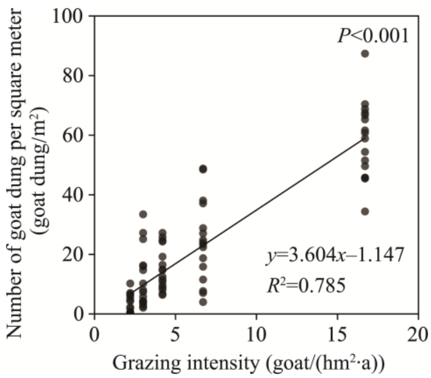

Fig. S5 Quantitative relationship between grazing intensity and the number of goat dung per square meter

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